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OPP Docket Telephone: (703)305-5805

SHERATON CRYSTAL CITY HOTEL
1800 JEFFERSON DAVIS HIGHWAY
ARLINGTON, VIRGINIA 22202
703-486-1111

ASSESSMENT OF ADDITIONAL SCIENTIFIC INFORMATION CONCERNING STARLINK™ CORN

CONSIDERATION BY EPA OF AVENTIS CROPSCIENCE TOLERANCE PETITION AND RELATED INFORMATION

The Panel has been provided with information and heard presentations on activities that have predominately taken place since this Panel met last November. These activities span a wide range of government, food industry, and pesticide company actions to characterize the allergenic risk from Cry9C protein in the human food supply. These activities have included efforts: to prevent further amounts of StarLink corn and corn expressing the Cry9C protein from entering the human food supply; to better characterize the exposure to Cry9C; to follow-up on individuals who reported potential allergic reaction from eating foods containing corn; and to develop methods to test for Cry9C antibodies in human blood.

On April 23, 2001, Aventis submitted a document to EPA which has been provided to the SAP and was made available to the public through EPA's web site and the Office of Pesticide Programs' public docket. EPA has requested that Aventis provide an overview of that material to this public meeting of the SAP. In this paper, EPA provides a brief summary of our review of that submission and other relevant information.

StarLink Corn Containment Programs:

USDA and Aventis have cooperated with a number of companies in the food production chain to contain the StarLink corn produced since 1998 when there was first, limited field production. A major effort by Aventis and USDA has gone to identifying and containing the 2000 StarLink crop and insuring that nearly all of that crop has been or will be directed to domestic animal feed or industrial uses. In addition, over 400 million bushels of Cry9C-commingled corn grain (probably mostly from 1999) have been identified and redirected to approved uses. As part of the

containment and redirection program, elevator operators, millers, and others have been testing for the presence of Cry9C protein using a lateral strip test developed by private industry and validated by the USDA's Grain Inspectors, Packers, and Shippers Association (GIPSA). USDA, the American Seed Trade Association, the National Corn Growers Association, and others have implemented a program to test the seed crop for 2001 to prevent Cry9C-positive seed corn from being planted and efforts will continue to test corn grain going to elevators and mills this year so that any grain testing positive for Cry9C protein will be redirected to appropriate uses.

Aventis has submitted a description of their StarLink Quality ✓Plan for Corn Dry Mills which includes testing using an enhanced version of the plan that FDA recommended on January 19, 2001 be followed for testing corn grain prior to dry milling. Overall this plan includes training by Aventis and calls for additional efforts to collect a representative sample of the container, maintaining half the sample for other testing, including doing positive controls during the tests, recording the results and maintaining the records, and allowing Aventis to audit the records. There is no indication in this description of the extent to which this program is in operation or if there will be some third party to audit this program.

StarLink Quality ✓Plan for Corn Dry Mills revealed that less than 5 % of the truckloads of grain had detectable levels (0.125%) of StarLink corn using the lateral flow strip test. EPA made adjustments to our expected exposures in the final version of the Wet Milling paper although EPA did not agree with Aventis in the concept of averaging residues within this distribution because most of the variability is driven by the different amounts of StarLink corn planted in different states. EPA does not believe that there is significant blending of corn from different regions of the country.

The Agency's estimates of the concentration of Cry9C in corn shipments to food processing facilities suggest that actual levels are significantly lower than 20 ppb. Depending on the methodology used, the Agency predicts average Cry9C residues in food corn of 0.34 to 8.0 ppb. These estimates are based on information provided by Aventis on the amount of unaccounted StarLink and buffer corn and on statistical analysis on the frequency of positive (5%) and negative (95%) detects in the Lateral Strip Flow monitoring program (Brassard, 2001b).

EPA believes that testing before milling and similar efforts have reduced dramatically the amount of Cry9C protein likely to be in the human food supply compared to the estimates developed last November. With continued testing, redirecting grain testing positive for Cry9C, and not selling seed testing positive for Cry9C, EPA estimates that Cry9C will essentially be gone from corn grain in 2 to 3 years and, considering the time it takes corn products to move through the channels of trade, gone from finished food products made from such corn in 4 to 5 years. See Brassard, 2001.

ELISA Assays to Detect Cry9C-Specific IgG and IgE Antibodies and Potential Follow-up

for Individuals Reporting Potential Allergic Reactions after Consuming Products Made with Corn:

Aventis reported on their efforts to develop ELISA tests for the presence of Cry9C-specific IgG and IgE antibodies and to run these tests on human, goat, and rabbit blood serum. FDA and CDC conducted a similar effort to develop an ELISA test for the presence of Cry9C-specific human IgE antibodies. While both test methodologies would be greatly improved with a positive control for Cry9C protein in human sera (i.e. IgE serum from Cry9C protein sensitized individuals), none is currently available. The Aventis work used human blood sera obtained from pre- or post-1998 samples and did not have the opportunity to use blood sera which FDA and CDC had from individuals who had reported potential allergic reactions to Cry9C protein. Unfortunately, Aventis did not test blood sera from individuals who were known to have contact with StarLink corn such as those who bagged the seed for sale.

The results of the Aventis tests indicate that neither serum samples from individuals with known allergies nor from other available human serum samples reacted to Cry9C antibodies. The Agency considers that the Aventis studies are still in the method development stage and add little to the overall information on the risk of allergenicity to Cry9C protein because of the lack of a positive Cry9C control. It is not possible to determine if any of the individuals whose blood serum was used for the tests performed by Aventis was ever exposed to the Cry9C protein.

Aventis recognizes the limitations of the ELISA blood serum tests in the absence of a positive control serum. In a letter to the Administrator, the company has recommended that a double-blind, placebo-controlled food challenge (DBPCFC) be conducted whether or not ELISA blood serum work resulted in positive responses to Cry9C protein. No such tests have yet been conducted.

Detection of Cry9C Protein in Products Using 100% StarLInk Corn which Had Been Dry Milled, Wet Milled, and Masa Processed:

Aventis had 14 food items made with 100% StarLink corn and a non-StarLink corn variety (Pioneer 3751) as a control to test for the presence or absence of the *cry9c* DNA and Cry9C protein in the finished food items. The data provided indicate that each of the food products made using 100% StarLink tested positive via PCR for *cry9c* DNA, except for the refined oil samples. None of the food products made with the non-StarLink corn showed the presence of *cry9c* DNA. These results suggest that at the very least, fragments of *cry9c* DNA remain intact through the processing described. As such, it appears that both the DNA extraction and PCR amplification methods described are adequate for detection of *cry9c* DNA. However, the presence of the DNA does not provide any indication of the effects of each processing method on Cry9C protein. It would not be possible to extrapolate that the presence of *cry9c* DNA alone indicates the presence of the Cry9C protein. PCR testing can allow for a determination that StarLink corn was used to make a particular product, but additional testing (immunological methods) is necessary to confirm the absence or presence of Cry9C protein.

Aventis used two ELISA-based protein detection systems to detect the Cry9C protein, one they developed and one developed by Envirologix Inc. The new Envirologix method is more sensitive that the lateral flow strip test currently used to identify corn grain containing Cry9C protein. A round robin validation of this test method has been reported to EPA by FDA, although EPA has not yet been able to review the raw data from this multi-laboratory validation exercise. Seven laboratories participated in the round robin validation using eight types of corn-based foods (starch, refined oil, soft tortillas, tortillas chips, corn flakes, corn puffs, corn muffins, and corn bread). The estimated level of quantification (LOQ) for the method was estimated to be 194 nanograms/gram protein and it was determined by FDA that the method is applicable to determine Cry9C protein in the eight types of corn-based foods at levels of $\geq$ 2 nanograms/gram or 2 ppb. This test method was used by Aventis to produce its revised dietary exposure assessment.

Revised Dietary Exposure Assessment:

Aventis' revised dietary exposure assessment was undertaken after Aventis conducted studies using 100 % StarLink corn in an effort to determine the impact of various types of processing on the levels of Cry9C protein in finished foods containing corn. EPA had previously recommended that such studies be conducted by the Aventis, especially after the SAP report from the February 2000 SAP meeting on Cry9C and non-digestible proteins was issued. The November SAP also recommended such studies to be conducted.

The new dietary assessment was conducted using Novigen's FARE™ (Food and Residue Evaluation) software. While EPA does not have access to this proprietary software, the information included in the model is the basis for the DEEM™ (Dietary Exposure Evaluation Model) software currently used by EPA. Aventis and Novigen provided EPA staff with a seminar on the FARE™ software. The FARE™ program can be used exactly like DEEM™, in that residue values for raw agricultural commodities (RACs) can be entered into the model, and exposure estimates generated. The FARE™ program also allows residue inputs to be entered for foods "as consumed." For the purpose of the revised dietary intakes assessment for Cry9C protein, the FARE™ program is considered to be more appropriate, since Cry9C protein levels were determined in processed foods.

The approach used by Aventis to determine estimated high end dietary intakes for the Cry9C protein is appropriate and actually may overestimate Cry9C dietary intakes for the following reasons:

- Conservative assumptions were used in translating Cry9C levels generated in the Aventis studies to other processed corn protein-containing foods.

- The analysis did not include an adjustment to account for the use of white corn (i.e., non-StarLink™) grain in food processing.

- Foods with non-detectable levels of Cry9C were assumed to contain the protein at the

limit of detection of the analytical method; EPA assessments typically incorporate non-detect residues at ½ the limit of detection.

At the same time, the Aventis submission assumes that the all grain used in making human foods is properly screened for the presence of StarLink and that, as a consequence of this screening, the grain contains no more than 0.125% StarLink. This assumption may be incorrect in that the screening may not be universally conducted in the same manner. To the extent that the screening procedure is conducted in a manner which is more sensitive, the levels of StarLink would be lower than 0.125%, and to the extent that the screening procedure is conducted improperly or in a less sensitive manner, it is possible that the grain could contain more than 0.125% StarLink. EPA does not have quantitative information to characterize the practices of screening corn for the presence of StarLink. All of the anecdotal information, as well as the infrequent detections of StarLink in finished foods, support a conclusion that proper screening is widespread, if not universal due to the voluntary nature of the program.

Conclusions:

EPA believes that the Aventis submission, taken with the other information presented from USDA, FDA, and CDC, supports that the exposure to Cry9C protein is significantly less than EPA estimated in its paper in November 2000. Moreover, we think the ongoing efforts of the growers, Aventis, the federal agencies, and facets of the food industry are greatly reducing, and will essentially eliminate, by 2004 or 2005, the amount of inadvertent Cry9C in US supplies of corn.

Brassard, D.W., 2001a. BEAD Estimate of Length of Time Corn Grain is Spent in Channels of Trade. EPA Memo from David W. Brassard to Janet Andersen and William Jordan. 2 pp.

Brassard, D.W., 2001b. Revised BEAD Review of "The Aventis CropScience StarLink Quality Plan for Corn Dry Mills". EPA Memo from David W. Brassard to Janet Andersen. 9 pp.

QUESTIONS FOR THE SAP

Exposure to Cry9C in Human Food:

At the November 2000 SAP meeting, EPA provided an exposure assessment for StarLink corn that was based on then available information. Specifically, EPA prepared an upper bound estimate of potential exposure to Cry9C protein as the consequence of the presence of StarLink in the human food supply. EPA used data on the consumption of food containing or made from corn, and data measuring the levels of Cry9C protein in corn grain. Notably, EPA assumed that the level of StarLink in finished foods was not reduced by processing of grain or by subsequent cooking.

EPA's exposure calculations also used information on the extent of planting of StarLink in 1999

and 2000, as well as information on grain handling practices, to produce high end estimates of the amount of StarLink corn that could be commingled with non-StarLink corn. These estimates did not take into account the various steps that have been taken since the fall of 2000 to limit the amount of Cry9C protein that could be present in the human diet. These steps include:

- the cancellation of the registration of StarLink, and the prohibition of future planting of stocks of StarLink seed;
- the Aventis Crop Science - USDA program to purchase StarLink™ corn (and any corn commingled with it) and to direct such corn to animal feed or industrial uses;
- USDA's program to assure that non-StarLink seed for the 2001 corn crop that tests positive for the Cry9C protein not be sold for planting; and
- the efforts of corn handlers, millers, and food processors to assure that corn grain is tested for the possible presence of the Cry9C protein and that quantities testing positive are redirected away from the human food chain.

In addition, since November, more refined data and new analyses have been developed to estimate the amount of the Cry9C protein that would be available in finished food products. A new analytical method has been developed by EnviroLogix that is capable of measuring the levels of Cry9C protein in finished foods. Aventis has also provided data on the impact of processing and cooking on the levels of Cry9C protein in various types of finished foods made from corn. Aventis has employed these data to produce new estimates of potential exposure to Cry9C protein. Finally, EPA has prepared its own estimates of exposure to Cry9C protein in human foods made from the wet milling of corn.

1. The performance of the EnviroLogix ELISA test for the determination of Cry9C protein processed corn-based foods was evaluated using eight types of corn-based foods in an interlaboratory study involving seven laboratories in the United States. The FDA report on the multi-laboratory validation of the new analytical method indicated that the method is applicable to the determination of the Cry9C protein in eight types of corn-based products at levels equal to or greater than 2 ppb. In light of this assessment, please comment on the utility of this method for assessing the concentration of Cry9C protein in processed corn based foods and specifically the use by Aventis of this method for their revised exposure estimates.

2. EPA has prepared a paper evaluating the impact of wet milling on the levels of Cry9C protein in human food products. Please comment on the levels of exposure to the Cry9C protein in the human diet likely to be encountered in food products as consequence of using human food fractions made from the wet milling of corn.

3. At the November 2000 meeting, the SAP reviewed the exposure assessment submitted by Aventis which estimated the possible levels of Cry9C protein that could be consumed by people eating food products made from corn if the corn contained any Cry9C protein. Aventis has submitted a revised exposure assessment which takes into account the new data estimating the levels of Cry9C protein that could survive processing, and thus occur in corn-based food products. Please comment on whether this updated assessment fairly and accurately depicts the

levels to which consumers may be exposed.

4. Assuming the measures taken to limit the amount of StarLink in the human food supply are continued and with your knowledge of how corn and food products made with corn move through the channels of trade, please comment on the duration and levels of detectable amounts (at ppb) of the Cry9C protein that are expected to be in the human food supply from:
a) StarLink ™ corn planted in 1998 through 2000; and
b) From other domestic sources that might contain the Cry9C protein, e.g., volunteer StarLink ™ corn and non-StarLink™ varieties that express the Cry9C protein.

Allergenic Hazard and Risk:

The potential for the Cry9C protein to elicit an allergic response has been the single human health endpoint of concern for Starlink corn. In its December, 2000 report to the Agency, the Scientific Advisory Panel (SAP) concluded that "... there is a medium likelihood that the Cry9C protein is a potential allergen..." The SAP went further to recommend a number of follow- up activities that would allow for a better informed characterization of the potential allergenic risk. These activities included: (1) collection of data on the presence of specific antibodies in individuals either who claim to have experienced adverse effects after consuming food that might have contained the Cry9C protein or who have significant occupational exposure to Starlink corn or corn products, and (2) monitoring of reports from the medical community for individuals who claim to have experienced adverse effects either after consuming food that might have been made from Starlink corn or from occupational exposure to Starlink corn.

5. FDA and CDC have been working together to investigate the adverse event reports submitted to FDA by people who claim to have had an allergic response following the ingestion of genetically modified corn products. One aspect of the investigation was to determine if these people were exposed and displayed an allergic response by the formation of serum antibodies to the foreign Cry9C protein. An FDA laboratory developed an enzyme linked immunosorbent assay (ELISA) method to detect these antibodies in the sera of the people who were potentially affected. Although there were no known Cry9C-allergic human serum samples to serve as true positive controls, the assay was able to detect reactions in sera from goats that had been purposefully sensitized against the Cry9C protein, and also to detect reactions to certain human allergens (e.g., cat, grass, peanut) in sera from humans with known allergies to these allergens.

Some of the individuals who claimed to have experienced an allergic reaction to the Cry9C protein following the ingestion of corn-based products kept samples of (or could identify) the products they ingested. FDA tested these foods for the presence of Starlink™ corn. Starlink™ corn DNA has not been detected in 10 of 11 food samples analyzed using the PCR method. The other sample of food, which tested positive using the PCR method, was not from the consumer's actual product, but from a different lot of the same product collected by FDA from a grocery store. In addition, the pCry9C protein was not detected is 9 (including the food sample that tested positive using the PCR method) of the 10 samples tested with the EnvioLogix ELISA method.

One of the 10 samples tested using the EnviroLogix method was inconclusive. There was no testing of one food sample using the EnviroLogix method because there was not enough of the remaining sample to conduct the test.

Given these circumstances, please comment on:

a) the ability of the test to detect Cry9C-specific antibodies;

b) the criteria used to designate test results as positive or negative, and the significance of positive and negative results obtained using this test;

c) the ability of the test to either identify or eliminate Cry9C as a potential cause of the allergic symptoms reported; and

d) the usefulness of the test, along with other information gathered in the FDA and CDC investigation, in evaluating whether an individual has experienced an allergic reaction to the Cry9C protein.

6. In the December, 2000 SAP report, after reviewing the information then available concerning the Cry9C protein, the Panel concluded that "... there is a medium likelihood that the Cry9C protein is a potential allergen based on the biochemical properties of the Cry9C protein itself..." The same report went on to state that "Given the current state of knowledge regarding allergens and the uncertainties of ascertaining the exact amounts of Cry9C in the food chain, this approach [collecting data on the presence of specific antibodies in individuals claiming exposure to Cry9C in food products] could provide 'hard evidence' as opposed to speculation on the question at hand." Since then, additional information concerning the potential allergenicity of the Cry9C protein has become available, including the FDA/CDC report issued on June 11, 2001, which provides information on the presence of Cry9C-specific antibodies in individuals claiming to have experienced an allergic reaction after eating corn-based foods. In light of the available information, what is the current Panel's view on the previous finding of that there is a "medium likelihood' that Cry9C protein is a human allergen? Please comment specifically on whether and how that view is significantly affected by your consideration of the June 11, 2001 reports from FDA and CDC.

7. In its December 1, 2000, report, the Panel concluded that "...the likely levels of Cry9C protein in the U.S. diet provide sufficient evidence of a low probability of allergenicity in the exposed population."

a) In light of the new information on the levels of Cry9C protein in the diet and the other available information concerning potential allergenicity, please comment on the overall probability that the likely levels in the US diet of Cry9C protein are sufficient to cause significant allergic reactions in a major identifiable subgroup of the exposed population. To the extent permitted by available information, please characterize the current level of potential risk in terms of the

proportion of the population likely to be affected and the nature and severity of potential effects.

b) If you conclude that it is probable that the expected levels of Cry9C protein are sufficient to cause significant allergic reactions in a major identifiable subgroup of the exposed population, please identify a level of Cry9C protein below which you would not expect significant reactions to occur in a major identifiable subgroup of the exposed population.

c) Based on your responses to questions 7 a) and 7 b), do you conclude that there appears to be a maximum level of Cry9C protein for which, if that level were found in corn grain and foods made from such grain, there would be a reasonable scientific certainty that exposure would not be harmful to public health? Please explain your answer.

Possible Need for Additional Data and Additional Public Health Measures:

8. In its December 2000 report, the SAP concluded "...the Agency should place ...priority on monitoring of reports from the medical community. The Panel felt that the medical community should be informed of the investigation into the allergenicity of Cry9C in corn products." Approximately 8 months have passed since that original recommendation and, given the materials that have been discussed at today's meeting, we ask the Panel to please comment on the value of implementing a program involving the medical community intended to detect instances in which individuals experienced allergic reactions to the ingestion of Cry9C protein in food. If the Panel still regards such a program as potentially valuable, then please comment on the scope and design of such a program.

9. In its December 2000 report, the SAP identified additional types of information that could improve EPA's ability to assess the potential allergenic risk to humans from Cry9C protein in the food supply. In response to the Panel recommendations, Aventis Crop Science and the Federal government have developed new information on the Cry9C protein which has been presented to the Panel today. Given all the information that we presently have, please characterize generally the adequacy of the existing scientific database to evaluate the allergenic risk of Cry9C and identify any additional information that would be feasible to generate and would be likely to change significantly the current assessment of the allergenic risk to humans from the Cry9C protein in the food supply.

10. From a public health perspective, please identify other measures, if any, beyond those currently being implemented that you consider feasible and necessary to reduce the likelihood that people would experience allergic reactions from ingestion of food containing Cry9C protein.

11. Are there any other comments on the science of this issue that EPA should consider or that the SAP panel would like to address?